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COMMENT

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# How BMP-2 induces EMT and breast cancer stemness through Rb and CD44?

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Tumor cells, originating from rare stem cells responsible for maintaining tumors, are organized hierarchically in certain malignancies<sup>1</sup>. Breast cancer stem cells (BCSCs, CD44<sup>+</sup>/CD24<sup>-</sup>) promote tumor progression and exhibit enhanced invasive properties that favor distant metastasis in patients with breast cancer<sup>2, 3</sup>.

In our previous study, we showed that bone morphogenetic protein (BMP)-2 inhibited cancer cell growth in vitro and in vivo by inducing G1 arrest and apoptosis in MDA-MB-231 and MCF-7 human breast cancer cell lines<sup>4</sup>. BMPs are known to be involved in metastatic progression and tumorigenesis of many types of cancer<sup>5</sup>, but functional studies have revealed contradictory roles of BMPs in both cancer promotion and inhibition<sup>6</sup>. Consequently, in our recent publication, we investigated the mechanism underlying the effect of BMP-2 on breast cancer metastasis using a comprehensive molecular approach in breast cancer cell lines and clinical breast cancer samples<sup>7</sup>.

We observed that rhBMP-2 induced epithelial–mesenchymal transition (EMT) in three breast cancer cell lines (MCF-7, MDA-MB-231, and a mouse breast cancer cell line 4T1) and enhanced the migratory and invasive capabilities of these cells both in vitro and in vivo. Next, we used the RT<sup>2</sup>Profiler PCR array (Qiagen, Hilden, Germany) to detect changes in the expression of 84 genes known to be associated with tumor metastasis. The most upregulated genes were *CD44* and *MMP11*, while the

most downregulated genes were *RB1* and *CDH1* (E-cadherin)<sup>7</sup>.

CD44, an alternatively spliced transmembrane protein, functions as a receptor for hyaluronan and a co-receptor for multiple receptor kinases associated with breast cancer<sup>8</sup>. *CD44* expression is essential for maintaining the cancer stem cell phenotype<sup>9</sup>. Immunocytochemistry assays showed that rhBMP-2 upregulated *CD44* expression and induced the redistribution of cellular CD44 to the leading edges and lamellipodia of MCF-7 cells. Using Smad4-siRNA silencing and the *CD44* promoter-luciferase reporter system, we further showed that rhBMP-2 upregulated *CD44* expression in MCF-7 cells via the conventional Smad-dependent signaling pathway. Binding of Smad4 to the SBE (Smad-binding element)-rich region of the *CD44* promoter activated *CD44* expression. BMP-2 also promoted the formation of tumor spheroids and increased the population of CD44<sup>+</sup>/CD24<sup>-</sup> cells in MCF-7 breast cancer cells. These observations suggest that rhBMP-2 enhances the stemness of breast cancer cells.

Rb (retinoblastoma) is a well-known tumor suppressor that initiates and maintains cell cycle arrest and modulates apoptosis. Functional loss of the *RB* contributes to aggressive tumor phenotype and induces EMT in breast cancer<sup>10</sup>. Unlike the Rb Ser567 phosphorylation-mediated and p-38 signaling pathway-activated induction of ubiquitin-dependent degradation of Rb in melanoma cells<sup>11</sup>, we observed that Rb was phosphorylated on Ser807/811 and subjected to ubiquitin-dependent degradation through a Smad-independent PI3K/AKT signaling pathway in BMP-2-activated breast cancer cells. Thus, we identified a unique mechanism of rhBMP-2-mediated Rb downregulation that promotes metastasis in MCF-7 cells.

Our results further showed that Rb reduction and activation of the PI3K/Akt pathway contributed partially to *CD44* upregulation. *CD44* expression was significantly upregulated in Rb-silenced cells than in control MCF-7

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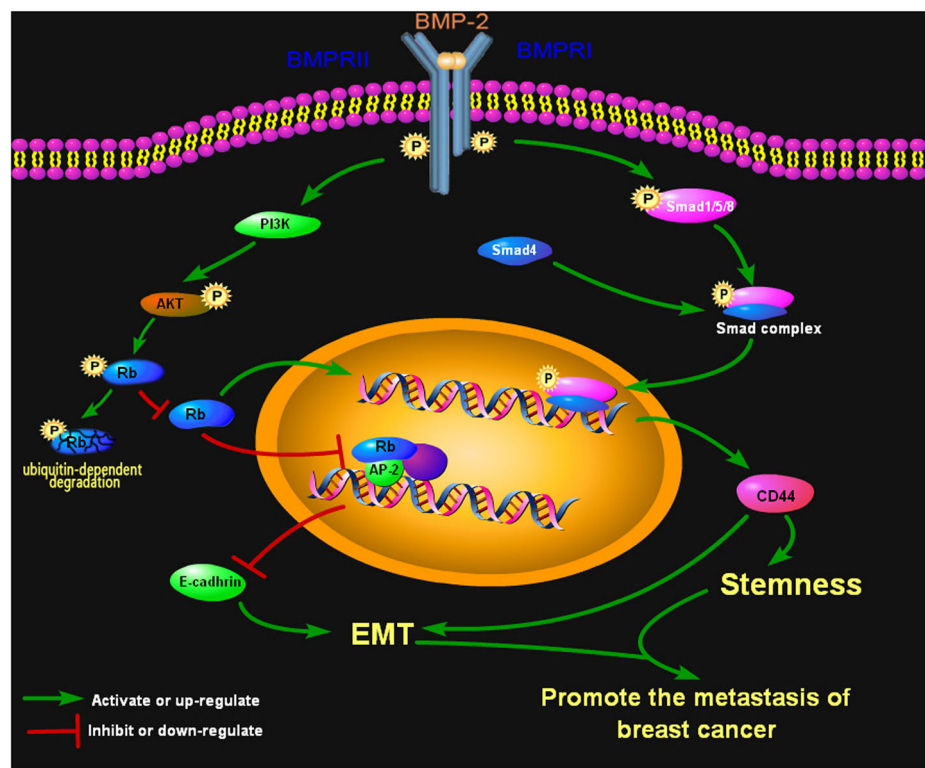
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**Fig. 1** This scheme depicts the signaling pathways via which BMP-2 induces EMT and stemness of breast cancer cells through Rb and CD44 and contributes to breast cancer metastasis. The PI3K/AKT and Smad signaling pathways are implicated in the BMP-2-mediated regulation of Rb and CD44, and a crosstalk exists between Rb and CD44 signaling pathways

cells. rhBMP-2-mediated CD44 upregulation was impaired in cells pretreated with PI3K and AKT inhibitors (LY294002 and MK-2206)<sup>7</sup>. These results were consistent with those of a recent study, which showed that CD44 expression was required for collective motility and metastatic progression initiated by loss of Rb function in breast cancer<sup>12</sup>. Our study also suggested that cross-talks between the Rb and CD44 pathways were required for BMP-2-dependent EMT and development of BCSCs.

Overall, this is the first study demonstrating that BMP-2 is a driving factor for promoting EMT and breast cancer stemness via Rb and CD44 signaling pathways (Fig. 1). Finally, we suggest that both PI3K/AKT and Smad signaling are involved in the rhBMP-2-mediated regulation of Rb and CD44 expression. Our in vitro and in vivo findings highlight the crucial roles of BMP-2, Rb, and CD44 in breast cancer metastasis, which may provide new strategies for determining the prognosis and treatment of advanced breast cancer.

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#### Competing interests

The authors declare that they have no competing financial interests.

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